Amendments to the Drawings

Attached herewith are Corrected Figures 5-8 and 11. The background of the Corrected Figures has been lighter than that of the original Figures 5-8 and 11.

Attachment: Replacement Sheet

REMARKS

Claim Amendments

Claims 23, 31, 33, 35, 37, and 42 have been cancelled.

Claims 22 and 41 have been amended to recite in part the subject matter of Claims 23, 32 and 34. Further support is found in Example 3, starting on page 27 (the disclosed plasmids $pZ_3klIL\text{-}1\beta$ and $pZ_3ppαIL\text{-}1\beta$ include the signaling sequences recited in Claim 22 as amended) and on page 10, lines 8-34.

Claims 22, 24, 26, 28-30, and 36 have been amended to correct their dependencies.

Claim 38, indicated as allowable by the Examiner, has been amended to recite the subject matter of its base Claim 22.

Amendments to Drawings

The Examiner required corrected drawings because the drawings that show blot results have dark background, making their interpretation difficult.

Applicants submit herewith Corrected Figures 5-8 and 11. The background of the Corrected Figures is lighter than that of the original Figures 5-8 and 11. Applicants believe that Corrected Figures 5-8 and 11 address the Examiner's objections.

Allowable Subject Matter

The Examiner stated that Claim 38 is objected to as dependent on a rejected claim, but would be allowable if re-written in an independent form.

Applicants recast Claim 38 in an independent form. Claim 38 should now be allowable.

Claim Rejection Under 35 U.S.C. §102

The Examiner rejected Claims 22 and 41 under 35 U.S.C. §102(b) as being anticipated by Radler *et al* (J. Gen. Microbiol. 1993, 139:495-500). The Examiner stated that Radler *et al.* disclose a methods of producing a protein comprising culturing a *Z. bailiii* strain, expressing and secreting a protein, and isolating the protein from the culture medium.

Applicants amended base Claims 22 and 41 to recite that the *Z. bailii* strain is transformed by a vector comprising a DNA sequence coding for a protein that is functionally linked to a signaling sequence selected from the group consisting of the signaling pre-sequence of the α-subunit of the K1 killer toxin of *Kluyveromyces lactis* and the signal sequence of the pre-pro α-factor of *Saccharomyces cerevisiae*.

Radler *et al.* do not disclosed the use of vectors that comprise DNA sequences that are functionally linked to signal sequences, much less to the specific signaling sequences recited in Claims 22 and 41 as amended. As such, the amended base claims are novel and non-obvious over Radler *et al.*

Reconsideration and withdrawal of the rejection are respectfully requested.

Claim Rejections Under 35 U.S.C. §103(a)

Claims 22-26, 28-31, 34, 36, 37, 39-42 are rejected under 35 U.S.C. §103(a) as being unpatentable over Brambilla *et al.* (WO 00/41477, "Brambilla") in view of Brake *et al.* (Proc. Natl. Acad. Sci. USA, 81, 4642-4646, 1984, "Brake"). Claim 27 is rejected under 35 U.S.C. §103(a) as being unpatentable over Brambilla, in view of Brake, and in further view of Jacobson *et al.* (WO 92/04461). Claim 32 is rejected under 35 U.S.C. §103(a) as being unpatentable over Brambilla in view of Brake, and in further view of Stark *et al.* (EMBO J., 5, 195-2002, 1986, "Stark").

Rejections of Base Claims 22 and 41 and Claim 32

Regarding base Claims 22 and 41, the Examiner stated that Brambilla discloses methods of production of a protein comprising the steps of culturing a *Z. bailii* strain, expressing a protein, and isolating a protein. The Examiner indicated that the difference between Brambilla and the instant claims is that Brambilla does not disclose that a protein is secreted and that a signal sequence is operably linked to the DNA encoding the protein. The Examiner stated that Brake discloses the use of the signal and pre-pro sequence of the gene encoding alpha-factor mating pheromone to direct secretion of heterologous genes in yeasts. The Examiner stated that it would have been obvious for one of ordinary skill to employ the expression system of Brake within the system of Brambilla to direct the secretion of proteins of interest.

Regarding Claim 32, the Examiner stated that Stark discloses the signal sequence of the alpha-subunit of the K1 killer toxin of *K. Lactis* and its function in secretion of proteins. The Examiner stated that it would have been obvious to one of ordinary skill to place the signal sequence of Stark in operable linkage with a protein of interest within the system of Brambilla.

Applicants respectfully submit that base Claims 22 and 41 as amended (which incorporate, in part, the subject matter of Claim 32) are non-obvious in view of the cited references.

Proper rejection under 35 U.S.C. §103(a) requires resolving a question of ''whether the improvement is more than the predictable use of prior art elements according to their established functions.'' (M.P.E.P. §2141(I), citing *KSR Int'l Co. v. Teleflex*, 550 US ___ at ___, (2007), 82 USPQ2d 1385 at 1396.) Applicants submits that the combination of cited references fails to render the base claims as amended obvious under the KSR guidelines because the use of the signal sequences recited in Claims 22 and 41 as amended confers unexpected and unpredictable advantages on the Applicants' expression system.

Applicants respectfully direct the Examiner attention to Example 3, starting on page 27, line 11 of the published application (WO 2004/042036). This example describes a comparison of the expression of a plasmid coding for Interleukin-1β (IL-1β) in *Z. bailii* and in *S. cerevisiae*.

In the first instance, both strains were transformed with a plasmid $pZ_3klIL\text{-}1\beta$ (page 27, line 14). This plasmid includes the leader sequence from the *K. lactis* killer toxin (page 17, lines 19-21). Example 3 describes that the transformed *Z. bailii* strain showed a surprisingly better secretory abilities than the similarly transformed *S. cerevisiae* strain (page 28, lines 18-27). Referring to FIG. 5, which shows the Western blot detection of the expressed IL-1β protein in either the minimal media (left column) or rich media (right column). Panels (b) show the results of IL-1β detection in cellular extracts (*i.e.* the non-secreted protein), while panels (c) and (d) show the results of IL-1β detection in the supernantant and media, respectively (*i.e.* the secreted protein). As can be seen, the signals corresponding to the protein secreted from the *Z. bailii* samples centers is significantly more intense than the signal corresponding to the non-secreted protein. In the *S. cerevisiae* samples, the intensities of the two signals have an opposite relationship: most of the expressed IL-1β remains non-secreted.

In the second instance, the *Z. bailii* and in *S. cerevisiae* were transformed by a plasmid $pZ_3ppαIL\text{-}1β$, which includes the pre-pro α-factor (the leader sequence of the alpha-factor pheromone) of *S. cerevisiae* (page 28, line 28-30). The results of the IL-1β expression are shown and compared in Figure 6(a) (see also text on page 29, lines 1-8). Figure 6(a) shows side-by-side comparisons of the Western blots signals corresponding to IL-1β expressed by the two strain in either rich (YPD, top four panels) or in minimal (YND, bottom two panels) media. The panels marked (i) and (iii) show the signal from cellular extracts (*i.e.* the non-secreted protein), while the panels marked (ii) and (iv) show the signal from supernatants (*i.e.* the secreted protein). As can be seen from the *difference* between the supernatants and the extracts, *Z. bailii* yeasts transformed with the $pZ_3ppαIL\text{-}1β$ plasmids secrete *more* protein than the similarly transformed *S. cerevisiae*.

The experimental data presented in Example 3 is unexpected, surprising and unpredictable. Furthermore, this data demonstrates that the protein expression process claimed in Claim 22 as amended and the strain claimed in Claim 41 as amended possess advantages over the expression systems disclosed in Brambilla, Brake and Stark, namely, high level of secretion of the expressed protein. As such, base Claims 22 and 41 as amended, as well as claims dependent thereon, including Claim 32, are non-obvious under the *KSR* guidelines.

Reconsideration and withdrawal of the rejections are respectfully requested.

Rejection of Claim 27

As stated above, Claim 27 is rejected under 35 U.S.C. §103(a) as being unpatentable over Brambilla, in view of Brake, and in further view of Jacobson *et al.* (WO 92/04461). The Examiner relies on the teachings in Jacobson *et al.* of a DNA sequence comprising at least 35 nucleotides of the SEQ ID NO: 69, which is claimed in Claim 27.

Applicants submit that Claim 27, which depends on base Claim 22 as amended through Claims 24 and 25, is non-obvious over the cited references for the same reasons as base Claim 22.

Reconsideration and withdrawal of the rejections are respectfully requested.

Rejections of Claims 23-26, 28-31, 34, 36, 37, 39 and 42

Claims 23, 31, 37, and 42 have been cancelled. With respect to the remainder of the above-listed claims, Applicants submit that these claims are non-obvious over cited references as dependent on base Claim 22 as amended, which is non-obvious as argued above.

Reconsideration and withdrawal of the rejections are respectfully requested.

Claim Rejections Under 35 U.S.C. §112

Claims 33, 35, and 37 stand rejected under 35 U.S.C. §112, first paragraph, as not enabled. The Examiner requested compliance with the Budapest Treaty with respect to biological deposits.

Without acquiescing to the Examiner's arguments, Applicants cancelled Claims 33, 35 and 37. Applicants do not disclaim the use of the plasmids and strains recited in the cancelled claims, or any other plasmids or strains within the scopes base Claims 22 and 41.

Reconsideration and withdrawal of the rejections are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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